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Detection of a Gene, vatD, Encoding an
Acetyltransferase Inactivating Streptogramin
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**Pending Claims**

1. A purified nucleic acid molecule comprising the DNA sequence of SEQ ID NO:2.

2. A purified nucleic acid molecule encoding an amino acid sequence comprising the sequence of SEQ ID NO:1.

3. A purified nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of any one of claims 1 or 2 under conditions of moderate stringency.

4. The purified nucleic acid molecule as claimed in claim 3, wherein said isolated nucleic acid molecule is derived by in vitro mutagenesis from SEQ ID NO:2 to NO 15.

5. A purified nucleic acid molecule degenerate from SEQ ID NOS:5, 6, 7, or 8 as a result of the genetic code.

6. A purified nucleic acid molecule, which encodes vatD polypeptide, an allelic variant of vatD polypeptide DNA, or a homolog of vatD polypeptide DNA.

7. A recombinant vector that directs the expression of a nucleic acid molecule selected from the group consisting of the purified nucleic acid molecules of claims 1, 2, 5, and 6.

8. A recombinant vector that directs the expression of a nucleic acid molecule of claim 3.

9. A recombinant vector that directs the expression of a nucleic acid molecule of claim 4.

10. A purified polypeptide encoded by a nucleic acid molecule selected from the group consisting of the purified nucleic acid molecules of claims 1, 2, 5, and 6.

11. A purified polypeptide according to claim 10 having a molecular weight of approximately 23,775 kDa as determined by SDS-PAGE.

12. A purified polypeptide according to claim 10 in non-glycosylated form.

13. A purified polypeptide encoded by a nucleic acid molecule of claim 3.

14. A purified polypeptide according to claim 13 in non-glycosylated form.

15. A purified polypeptide encoded by a nucleic acid molecule of claim 4.

16. A purified polypeptide according to claim 15 in non-glycosylated form.

17. Purified antibodies that bind to a polypeptide of claim 10.

18. Purified antibodies according to claim 17, wherein the antibodies are monoclonal antibodies.

19. Purified antibodies that bind to a polypeptide of claim 13.

20. Purified antibodies according to claim 19, wherein the antibodies are monoclonal antibodies.

21. Purified antibodies that bind to a polypeptide of claim 15.

22. Purified antibodies according to claim 21, wherein the antibodies are monoclonal antibodies.

23. A host cell transfected or transduced with the vector of claim 7.

24. A method for the production of vatD polypeptide comprising culturing a host cell of claim 23 under conditions promoting expression, and recovering the polypeptide from the culture medium.

25. The method of claim 24, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

26. A host cell transfected or transduced with the vector of claim 8.

27. A method for the production of vatD polypeptide comprising culturing a host cell of claim 26 under conditions promoting expression, and recovering the polypeptide from the culture medium.

28. The method of claim 27, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

29. A host cell transfected or transduced with the vector of claim 9.

30. A method for the production of vatD polypeptide comprising culturing a host cell of claim 29 under conditions promoting expression, and recovering the polypeptide from the culture medium.

31. The method of claim 30, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

32. The plasmid deposited at CNCM under the Accession Number I-2247.

33. An immunological complex comprising a vatD polypeptide and an antibody that specifically recognizes said polypeptide.

34. A method of detecting a bacterium in a biological sample that harbors a polynucleotide sequence according to claim 1, said method comprising the steps of:

a) contacting bacterial DNA of the biological sample with a primer or a probe according to claim 1 or 3 to 6, which hybridizes with a nucleotide sequence encoding resistance to streptogramins;

b) amplifying the nucleotide sequence using said primer or said probe; and

c) detecting a hybridized complex formed between said primer or probe and the DNA.

35. A kit for detecting a bacterium that is resistant to a streptogramin and harbors a polynucleotide sequence according to claim 1, said kit comprising:

a) a polynucleotide probe according to claim 19 or 20; and

b) reagents to perform a nucleic acid hybridization reaction.

36. A kit for detecting a bacterium that is resistant to a streptogramin and harbors a polynucleotide sequence according to claim 2, said kit comprising:

a) a polynucleotide probe according to claim 19 or 20; and

b) reagents to perform a nucleic acid hybridization reaction.

37. A method of screening an active antibiotic for treating a Gram-positive bacterial infection, comprising the steps of:

a) contacting the antibiotic with Gram-positive bacteria that are resistant to a streptogramin and contain a polynucleotide sequence according to claim 1; and

b) determining the activity of the antibiotic on the bacteria.

38. A method of screening for active synthetic molecules capable of penetrating into a bacteria of the enterococcus family, wherein an inhibiting activity of the molecules is tested on at least a polypeptide encoded by a polynucleotide sequence according to claim 1, the method comprising the steps of:

a) contacting a sample of said active molecules with the bacteria;

b) testing the capacity of the active molecules to penetrate into the bacteria and the capacity of inhibiting a bacterial culture at various concentration of the molecules; and

c) choosing the active molecule that provides an inhibitory effect of at least 80% on the bacterial culture compared to an untreated culture.

39. An in vitro method of screening for active molecules capable of inhibiting a polypeptide encoded by a polynucleotide sequence according to claim 1, said method comprising the steps of:

a) contacting the active molecules with said polypeptide;

b) testing the capacity of the active molecules, at various concentrations, to inhibit the activity of the polypeptide; and

c) choosing the active molecule that provides an inhibitory effect of at least 80 % on the activity of the said polypeptide.

40. A method of detecting a bacterium in a biological sample that harbors a polynucleotide sequence according to claim 2, said method comprising the steps of:

a) contacting said sample with an antibody according to claim 17 that recognizes a polypeptide encoded by said polynucleotide sequences; and

b) detecting a complex formed between the antibody and the polypeptide.

41. A diagnostic kit for in vitro detection of a bacterium harboring the polynucleotide sequences according to claim 2, said kit comprising:

a) a predetermined quantity of monoclonal or polyclonal antibodies according to claim 17;

b) reagents to perform an immunological reaction between the antibodies and a polypeptide encoded by said polynucleotide sequences; and

c) reagents for detecting a complex formed between the antibodies and the polypeptide encoded by said polynucleotide sequences.